

AMENDMENTS

Amendments to the Claims:

This listing of claims will replace all prior versions and listings of claims in the application:

1-21. (Cancelled)

22. (New) A method for the synthesis and recovery of a secreted, biologically active heterologous (non-yeast) heteromultimeric polypeptide comprising at least two non-identical subunit polypeptide chains, the method comprising:

(i) producing one or more stable diploid *Pichia* cells by mating or spheroplast fusion of haploid *Pichia* cells under conditions yielding one or more diploid *Pichia* cells, wherein said diploid cells comprise at least one expression construct which encode for at least two non-identical subunit polypeptide chains, and which stable diploid *Pichia* cells are capable of the assembly, expression and secretion of said heteropolymeric polypeptide which is comprised of said least two non-identical subunit polypeptide chains into a culture medium when cultured in said medium under appropriate culture conditions;

(ii) culturing said diploid *Pichia* cells, or diploid progeny thereof, in a culture medium under conditions resulting in the expression, assembly and secretion of said biologically active heteromultimeric polypeptide in the culture medium; and

(iii) recovering the resultant heteromultimeric polypeptide from the culture medium.

23. (New) The method of claim 22, wherein said mating or spheroplast fusion is effected by mating or fusing a first haploid *Pichia* cell containing a first expression construct, said first expression construct comprising nucleic acid sequences encoding for the expression of at least one subunit of said heteromultimeric polypeptide, operably linked to a first yeast promoter; and a second haploid *Pichia* cell containing a second expression construct, said second expression construct comprising nucleic acid sequences encoding for the remaining subunit(s) of said heteromultimeric polypeptide, operably linked to a second yeast promoter.

24. (New) The method according to claim 22, wherein said *Pichia* cells are selected from *Pichia pastoris*, *Pichia methanolica*, and *Pichia angusta*.

25. (New) The method according to claim 24, wherein said *Pichia* cells are *Pichia pastoris*.
26. (New) The method of claim 22, wherein the heteromultimeric polypeptide is an antibody or an antigen binding antibody fragment.
27. (New) The method of claim 22, wherein said expression constructs are integrated into the genome of said diploid *Pichia* cells.
28. (New) The method of claim 22, wherein said expression constructs are contained on extrachromosomal elements.
29. (New) The method of claim 22, wherein the first or second promoters are constitutive.
30. (New) The method of claim 22, wherein the first or second promoters are inducible.
31. (New) The method of claim 22, wherein the diploid yeast cells are grown in a production media.
32. (New) The method of claim 30, wherein said production media is a minimal media.
33. (New) The method of claim 31, wherein said minimal media lacks selective agents.
34. (New) The method of claim 31, wherein said minimal media lacks pre-formed amino acids or other complex biomolecules.
35. (New) The method of claim 22, wherein said diploid *Pichia* cells are grown to a high cell density.
36. (New) The method of claim 35, wherein said high cell density is at least 50 g/L.
37. (New) The method of claim 36, wherein said high cell density is at least 100 g/L.
38. (New) The method of claim 37, wherein said high cell density is at least 300 g/L.
39. (New) The method of claim 38, wherein said high cell density is at least 400 g/L.
40. (New) The method of claim 39, wherein said high cell density is at least 500 g/L.

41. (New) The method of claim 22, wherein said diploid *Pichia* cells are grown under conditions resulting in levels of said biologically active heteromultimeric polypeptide in the culture medium which are at least 50 mg/L.
42. (New) The method of claim 41, wherein said diploid *Pichia* cells are grown under conditions resulting in levels of said biologically active heteromultimeric polypeptide in the culture medium which are at least 100 mg/L.
43. (New) The method of claim 42, wherein said diploid *Pichia* cells are grown under conditions resulting in levels of said biologically active heteromultimeric polypeptide in the culture medium which are at least 500 mg/L.
44. (New) The method of claim 43, wherein said diploid *Pichia* cells are grown under conditions resulting in levels of said biologically active heteromultimeric polypeptide in the culture medium which are at least 1000 mg/L.
45. (New) The method of claim 22, wherein the diploid *Pichia* cells maintain high levels of expression of said heteromultimeric polypeptide after culturing for at least 20 doublings.
46. (New) The method of claim 45, wherein the diploid *Pichia* cells are maintain high levels of expression of said heteromultimeric polypeptide after culturing for at least 50 doublings.
47. (New) The method of claim 46, wherein the diploid *Pichia* cells maintain high levels of expression of said heteromultimeric polypeptide after culturing for at least 100 doublings.
48. (New) The method of claim 22, wherein at least 99% of said diploid *Pichia* cells comprise said expression constructs after culturing for at least 20 doublings.
49. (New) The method of claim 48, wherein at least 99% of said diploid *Pichia* cells comprise said expression constructs after culturing for at least 50 doublings.
50. (New) The method of claim 49, wherein at least 99% of said diploid *Pichia* cells comprise said expression constructs after culturing for at least 100 doublings.

51. (New) The method of claim 22, wherein the diploid *Pichia* cells express the heteromultimeric polypeptide at a level of expression which is reduced by not more than 20% relative to the starting level of expression after culturing for at least 20 doublings
52. (New) The method of claim 51, wherein the diploid *Pichia* cells express the heteromultimeric polypeptide at a level of expression which is reduced by not more than 20% relative to the starting level of expression after culturing for at least 50 doublings.
53. (New) The method of claim 52, wherein the diploid *Pichia* cells are express the heteromultimeric polypeptide at a level of expression which is reduced by not more than 20% relative to the starting level of expression after culturing for at least 100 doublings.
54. (New) The method of claim 31, wherein the diploid *Pichia* cells express the heteromultimeric polypeptide at a level of expression which is reduced by not more than 10% relative to the starting level of expression.
55. (New) The method of claim 31, wherein the diploid *Pichia* cells express the heteromultimeric polypeptide at a level of expression which is reduced by not more than 5% relative to the starting level of expression.
56. (New) The method of claim 22, wherein said culture containing said diploidal *Pichia* cells is grown at a temperature of not more than 22°C.
57. (New) A culture medium containing a stable diploid *Pichia* culture according to claim 22, wherein the culture medium comprises expression levels of said biologically active heteromultimeric polypeptide which are at least about 50 mg/liter.
58. (New) A culture medium containing a stable diploid *Pichia* culture according to claim 22, that expresses said heteromultimeric polypeptide into a culture medium, wherein the cell density of said *Pichia* diploid cells in said culture are at least about 50 g/L.